At scales greater than 500 L the refold buffer was prepared and allowed to equilibrate at approximately 5° C. to achieve a stable oxygen level in the solution (typically 50% to 70% dissolved oxygen, relative to air saturation). Once the refold mixture was formed, the vessel was sealed and the incubation period was initiated.

The protein concentration of the refold mixture was 6 g/L, which is a four-fold enhancement over the recovery of 1.5 g/L obtained using a method other than the method described in this Example. Overall annual process productivity, in one specific manufacturing facility, was calculated to be increased by >930% due to increased volumetric efficiency in the existing facility tanks.

Example 5

Effect of Thiol-Pair Oxidation State on Disulfide Pairings

FIGS. **1***a*-**1***f* demonstrate that as the thiol-pair ratio is forced to a more oxidizing state (lower thiol-pair ratio), a higher proportion of product species have oxidized amino acid residues and mixed disulfide forms. As the thiol-pair ratio is driven to a more reductive state (higher thiol-pair ratio), this results in lower levels of oxidized amino acid variant species and higher levels of product species with incorrect disulfide pairings or unformed disulfide bonds. As the overall thiol-pair buffer strength is modified, the corresponding optimal thiol-pair ratio is shifted. This effect is similar to how buffer strength modulates the sensitivity of pH to acid and base additions in a buffered solution.

An optimal balance of species was attainable. As shown in FIGS. **1***a*-**1***f*, there is a clear relationship between thiol-pair buffer strength and thiol-pair ratio that can be identified to maintain the optimal species balance and thus facilitate efficient refolding of low solubility proteins. The ability to control product variant species, such as incorrectly disulfide-bonded species and misfolded species, via modulation of the thiol-pair ratio and thiol-pair buffer strength, enables efficient, effective and reliable subsequent purification processes.

Example 6

Effect of Non-Aerobic Conditions on Refolding Efficiency

FIGS. **2** and **3** demonstrate that when the thiol-pair buffer strength is selected appropriately, taking into account the protein concentration and number of cysteine residues in the protein, the sensitivity to external influences, such as oxygen, is significantly reduced. This allows for a non-aerobic refolding condition that is significantly easier to transfer between scales and reactor configurations.

FIG. **2** compares the RP-HPLC analytical species distribution between a 15 L-scale refold and a 20 mL-scale refold under several environmental conditions. For Condition 1 (the trace labeled "1" in FIG. **2**), the solubilization chemicals and solutions were dispensed in air and the refold mixture was incubated in air. In Condition 2 solubilization chemicals and solutions were dispensed in air and incubated under nitrogen headspace. In Conditions 3-7 solubilization chemicals and solutions were dispensed under nitrogen overlay conditions and in conditions 3, 5, 6, and 7 solubilization chemicals and solutions were incubated under nitrogen. In Condition 7, the refold solution was also stripped of nitrogen prior to combination with the solubilization solution. In Condition 4 the solubilization chemicals and solutions were incubated under ambient air conditions.

The results shown in FIG. **2** demonstrate that the conditions under which the solubilization chemicals and solutions were dispensed or incubated in the presence of air (i.e., Conditions 1, 2, and 4) do not achieve results that are comparable to the larger-scale control. In Conditions 1, 2 and 4, increased formation of oxidized species (pre-peaks) are observed. The pre-peaks are indicated by arrows in the panels for Conditions 1, 2 and 4.

FIG. **3** compares the RP-HPLC analytical results of an identified condition, achieved as described in Example 2, at 1 L-scale and 2000 L-scale. In this figure, essentially no difference in the distribution of species is detectable. Taken together, FIGS. **2** and **3** demonstrate that when aeration is carefully controlled, the small-scale refold reactions are more predictive of those expected upon scale-up of the refold reaction, facilitating the implementation of large-scale protein refolding processes.

What is claimed is:

1. A method of refolding proteins expressed in a non-mammalian expression system, the method comprising:

contacting the proteins with a preparation that supports the renaturation of at least one of the proteins to a biologically active form, to form a refold mixture, the preparation comprising:

at least one ingredient selected from the group consisting of a denaturant, an aggregation suppressor and a protein stabilizer;

an amount of oxidant; and

an amount of reductant,

wherein the amounts of the oxidant and the reductant are related through a thiol-pair ratio and a thiol-pair buffer strength,

wherein the thiol-pair ratio is in the range of 0.001-100; and

wherein the thiol-pair buffer strength maintains the solubility of the preparation; and

incubating the refold mixture so that at least about 25% of the proteins are properly refolded.

2. The method of claim **1**, wherein the refold mixture has a protein concentration in a range of 1-40 g/L.

3. The method of claim **1**, wherein the refold mixture has a protein concentration of 2.0 g/L or greater.

4. The method of claim **1**, wherein the thiol-pair buffer strength is 2 mM or greater.

5. The method of claim **1**, wherein the thiol-pair buffer strength is increased proportionally to an increase in a total protein concentration in the refold mixture.

6. The method of claim **1**, wherein the thiol-pair buffer strength is decreased proportionally to a decrease in a total protein concentration in the refold mixture.

7. The method of claim **1**, wherein the at least one of the proteins is a complex protein.

8. The method of claim **1**, wherein the thiol-pair ratio is calculated according to the following equation:

$$\frac{[\text{the reductant}]^2}{[\text{the oxidant}]}.$$

9. The method of claim **1**, wherein the thiol-pair buffer strength is calculated according to the following equation:

$$2[\text{the oxidant}]+[\text{the reductant}].$$